

Fig. 1A

Local DNA opening by PNA creates activated nucleation site for both RecA-coated complementary single-stranded probes

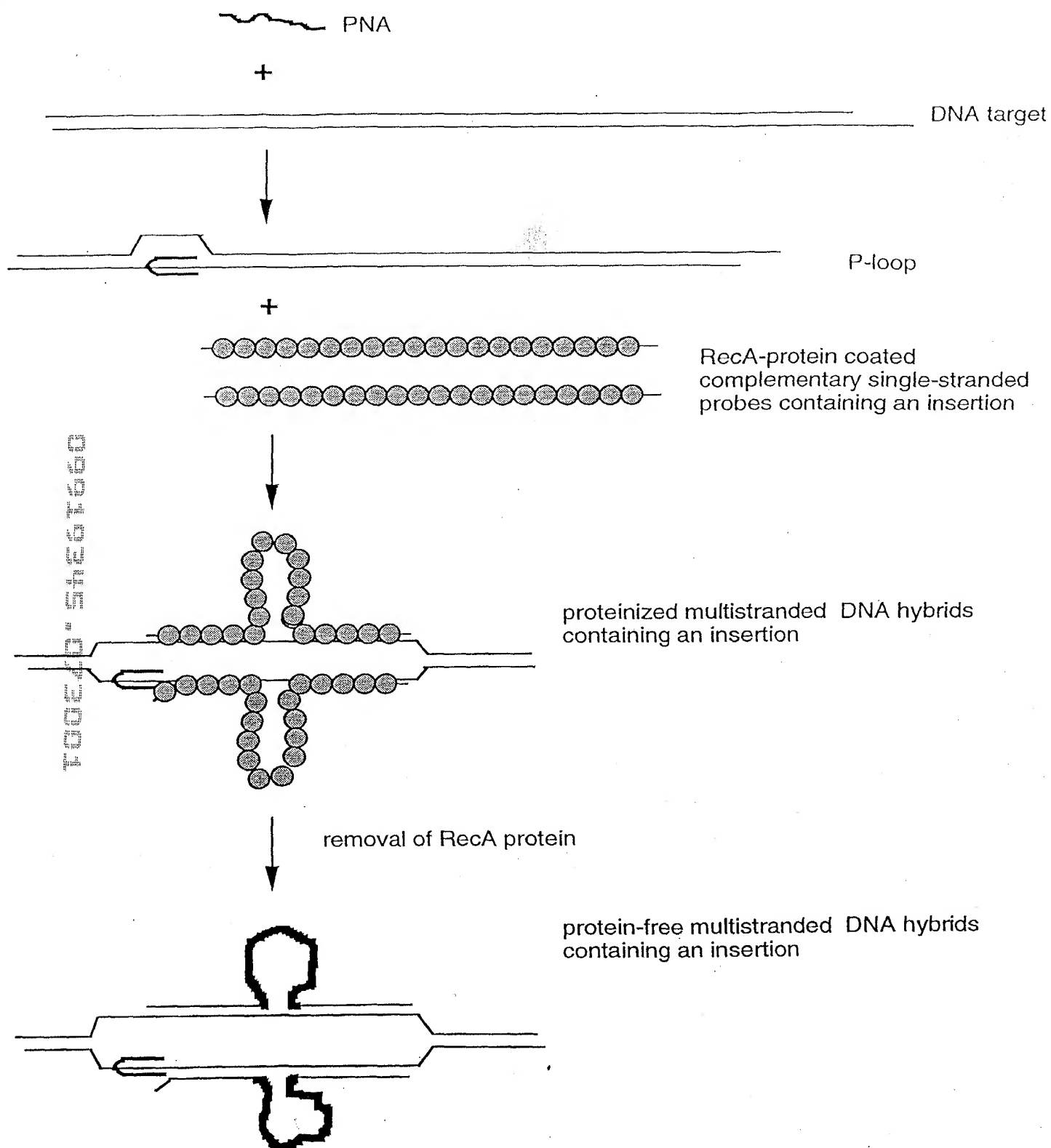


Fig 1B: Stabilization of single D-loop hybrids by analog probes.

When the PNA binding site is within the probe-target hybrid, PNA can stabilize single D-loops by trapping the strand exchange process.

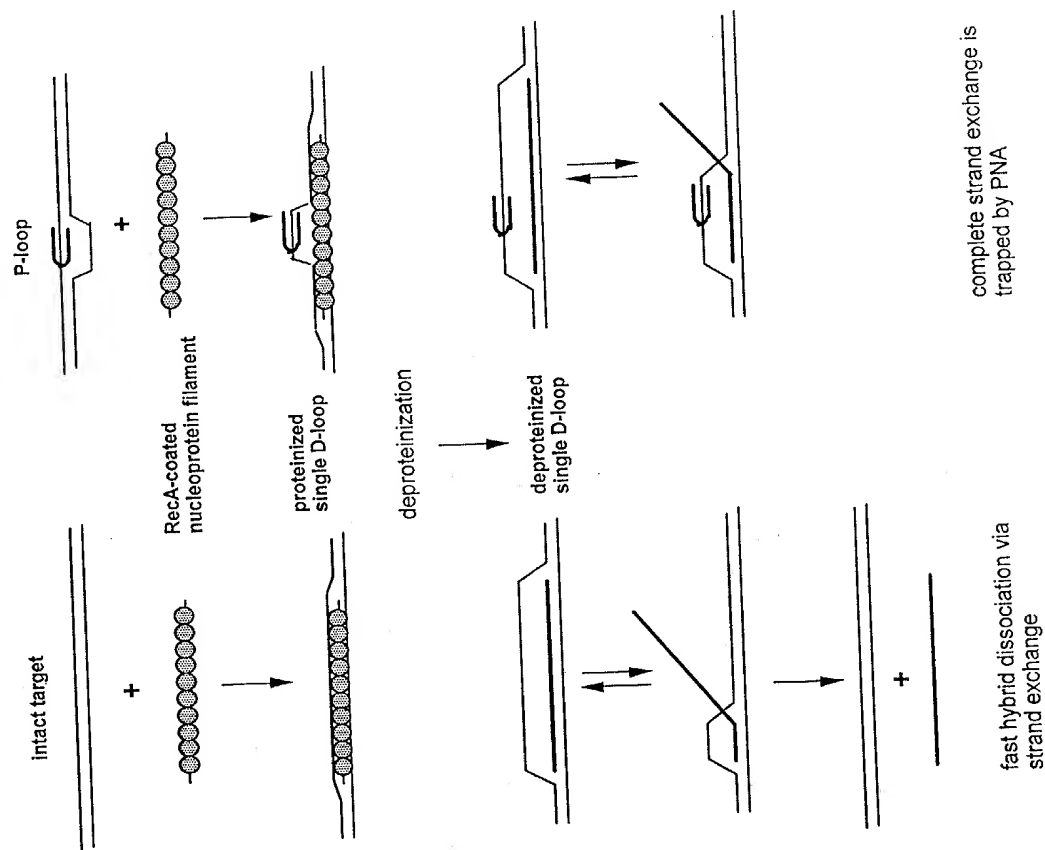


Fig. 1C

PNA activates the binding of the second coming RecA-coated single-stranded probe via stabilization of the "opened" state of the D-loop formed by the the first coming RecA-coated single-stranded probe

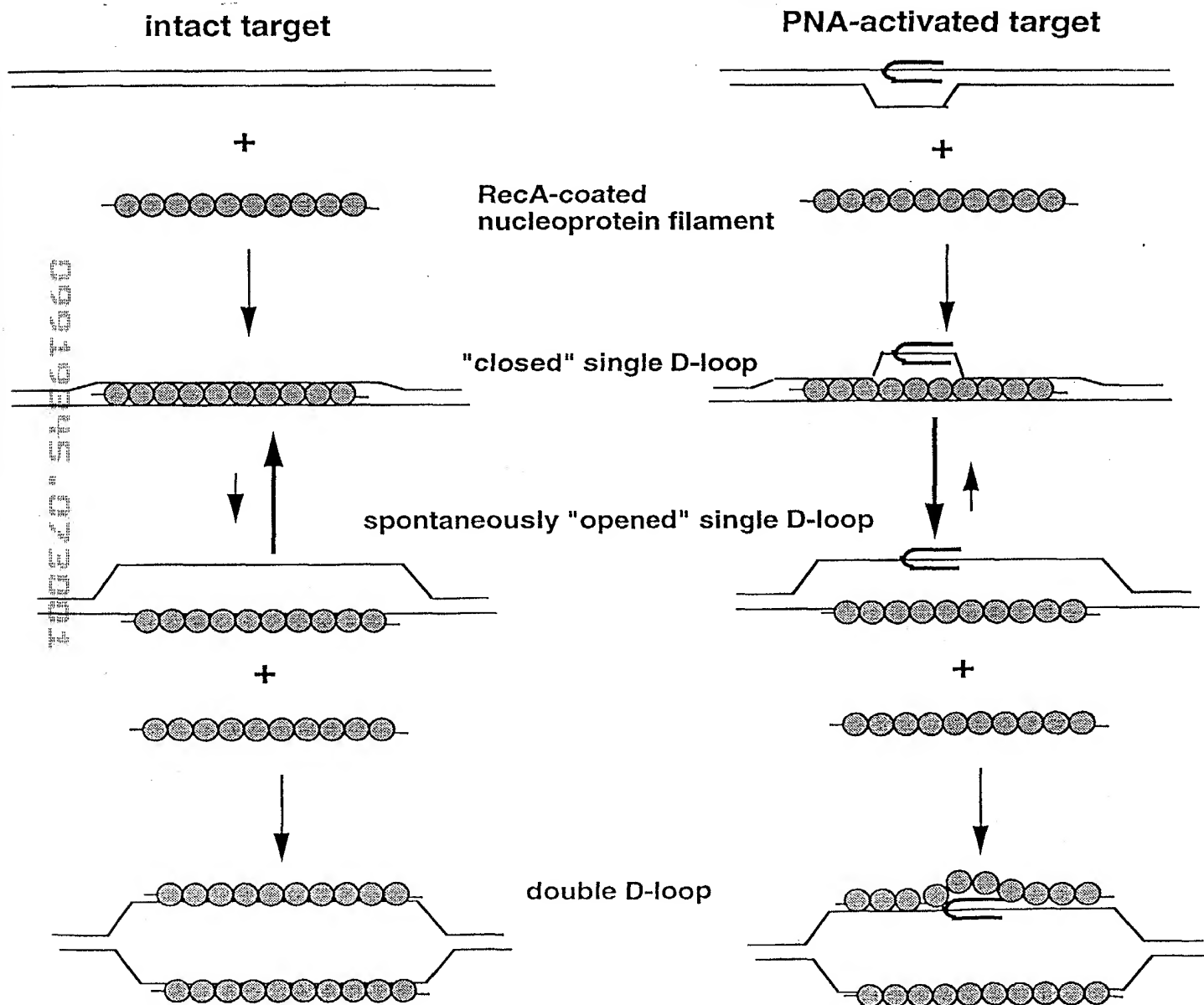


Fig. 2

PNA-directed double-stranded break in the target DNA followed by homologous DNA targeting

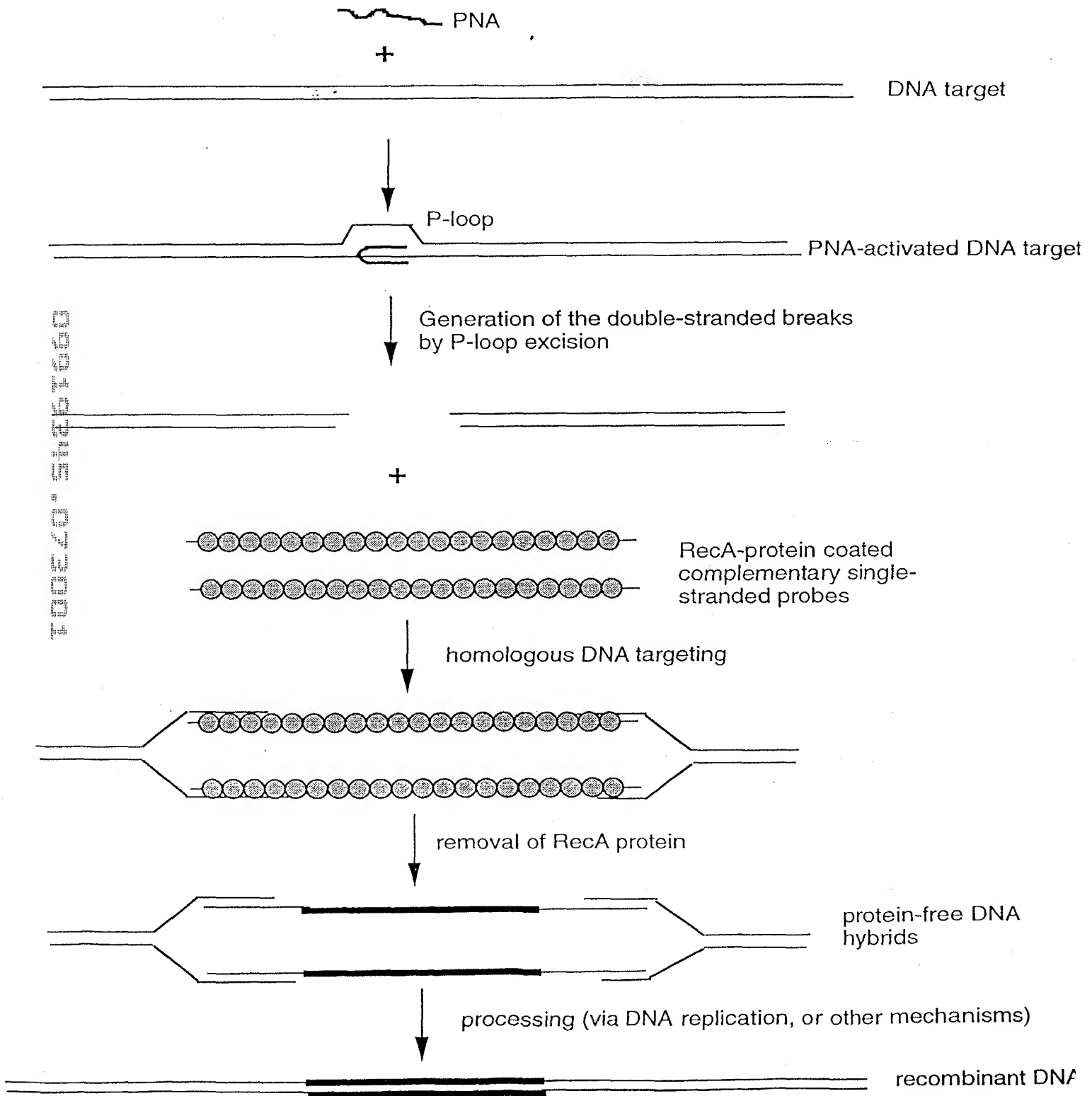


Fig. 3A Processing of the hybrids by strand excision followed by DNA repair

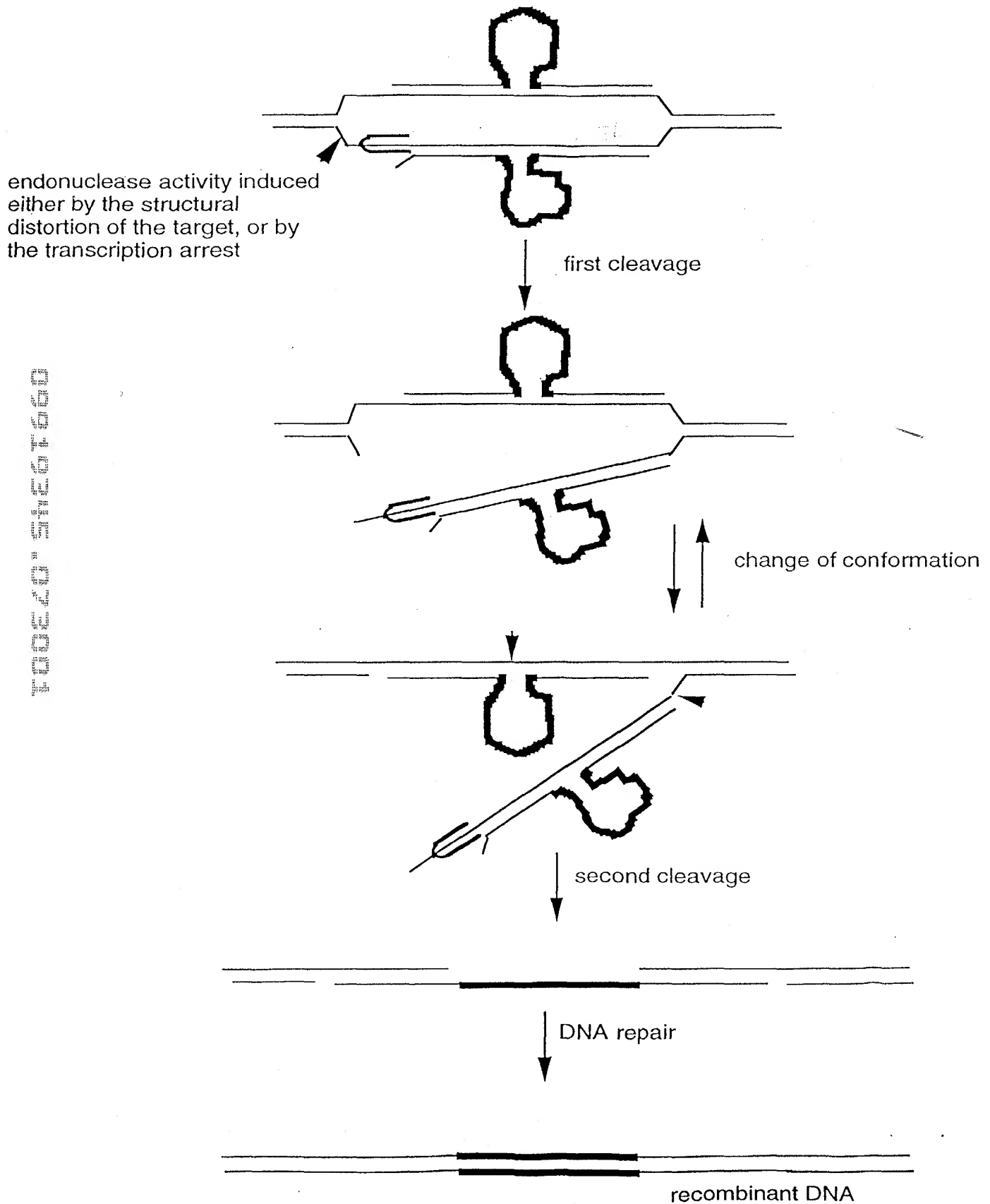


Fig.3B

Hybrid processing mediated by target DNA replication
when the PNA site is outside the heterologous insert
site

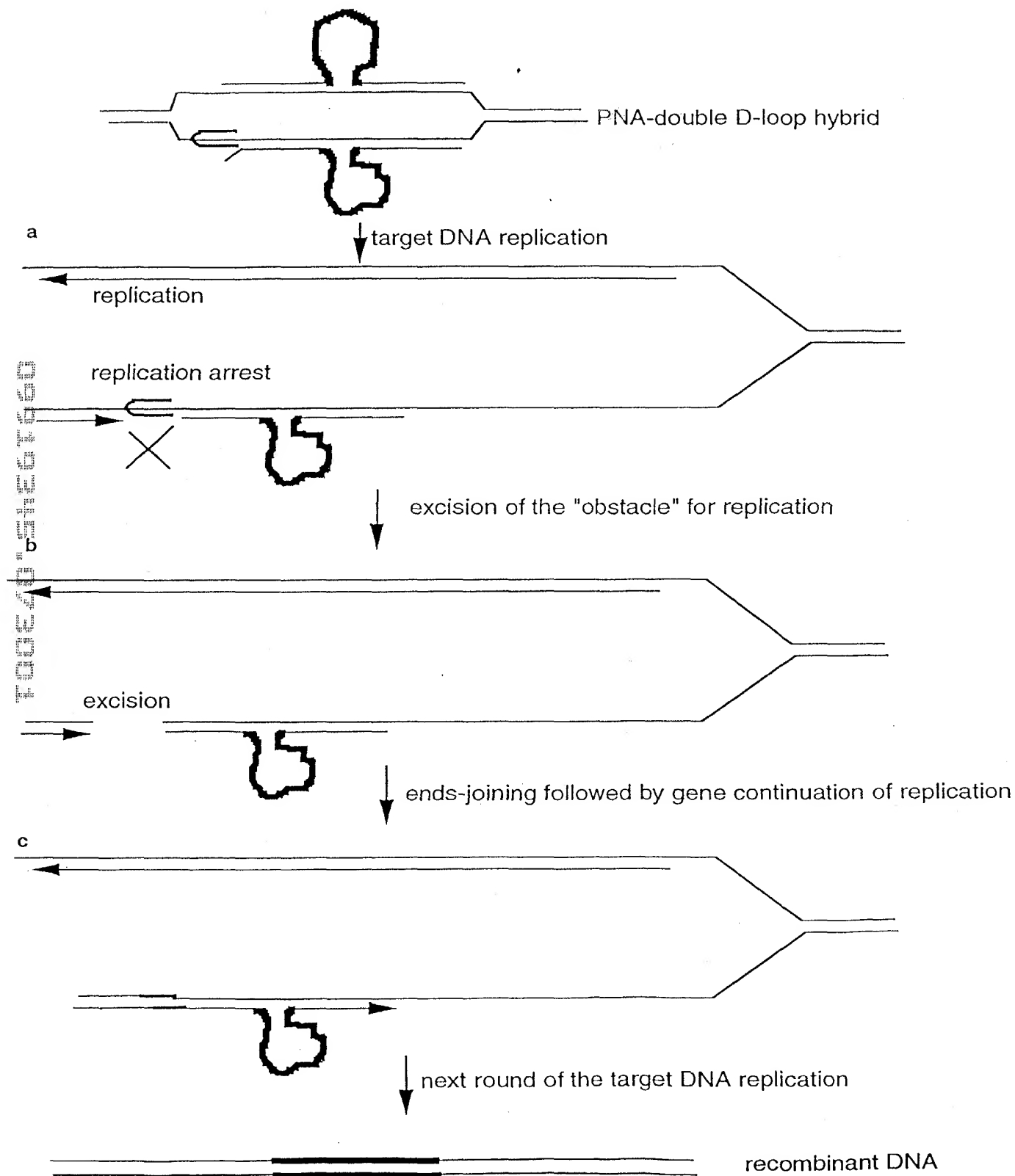


Fig. 3C Hybrid processing mediated by target DNA replication when the PNA site is inside the heterologous insert site

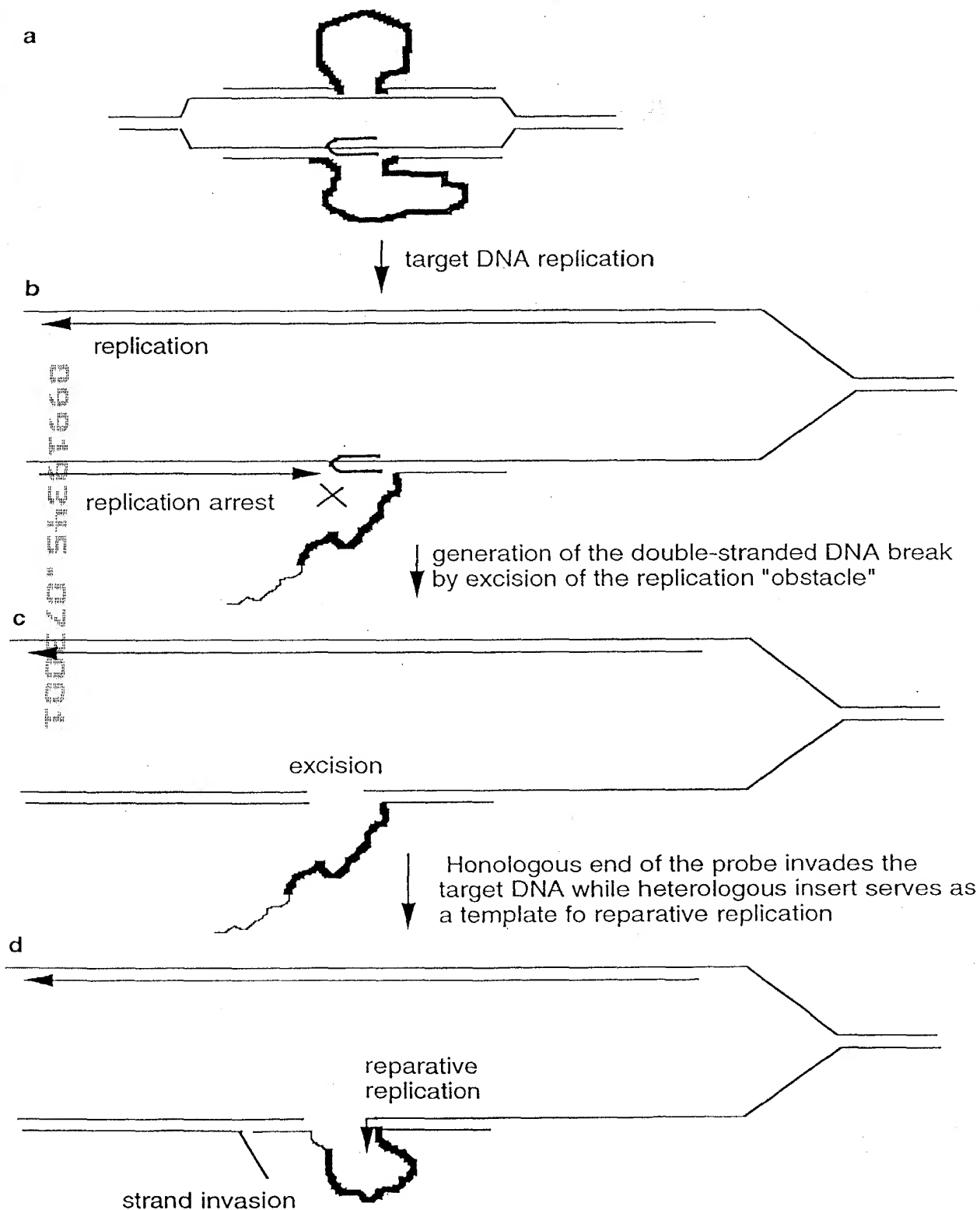


Fig 3C contd.

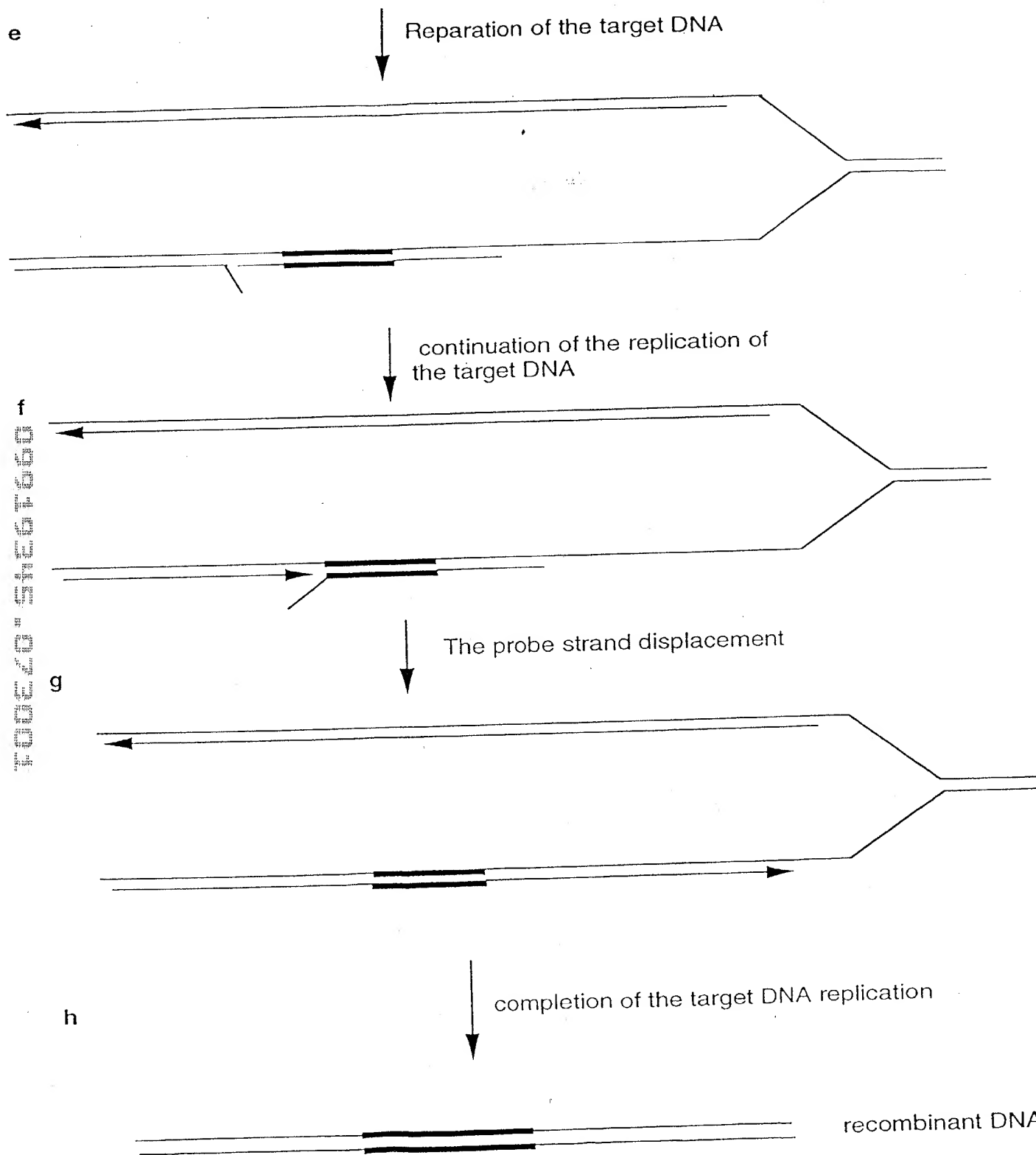
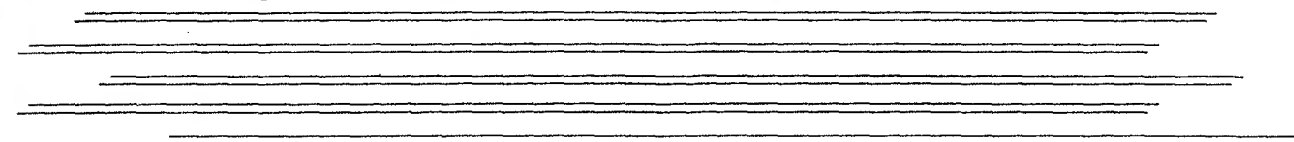
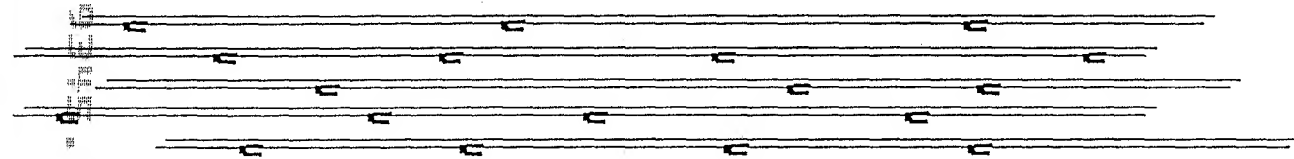


Fig.4. Cloning of linear (including genomic) DNA fragments mediated by PNA activated homologous DNA targeting

restriction fragments of genomic DNA



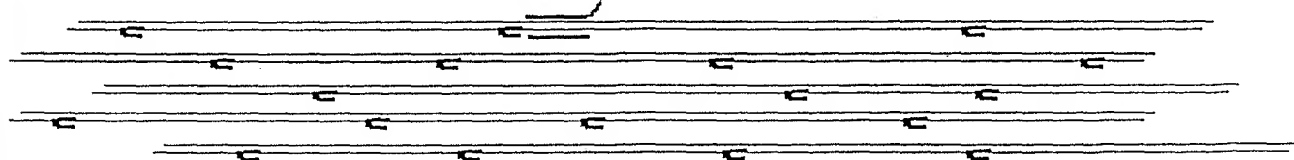
⌵ PNA (or mixture of PNAs) which bind approximately every 100-200 bp, including sites adjacent (or overlapping) with the target gene



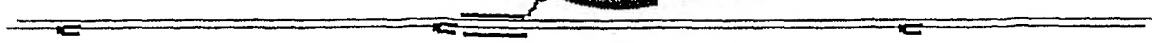
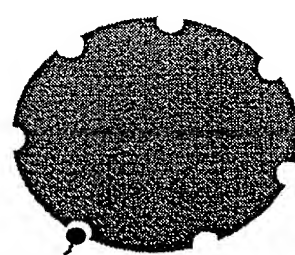
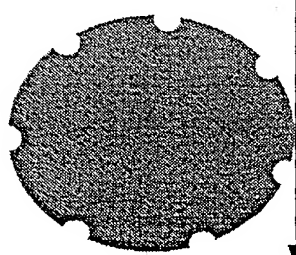
biotin

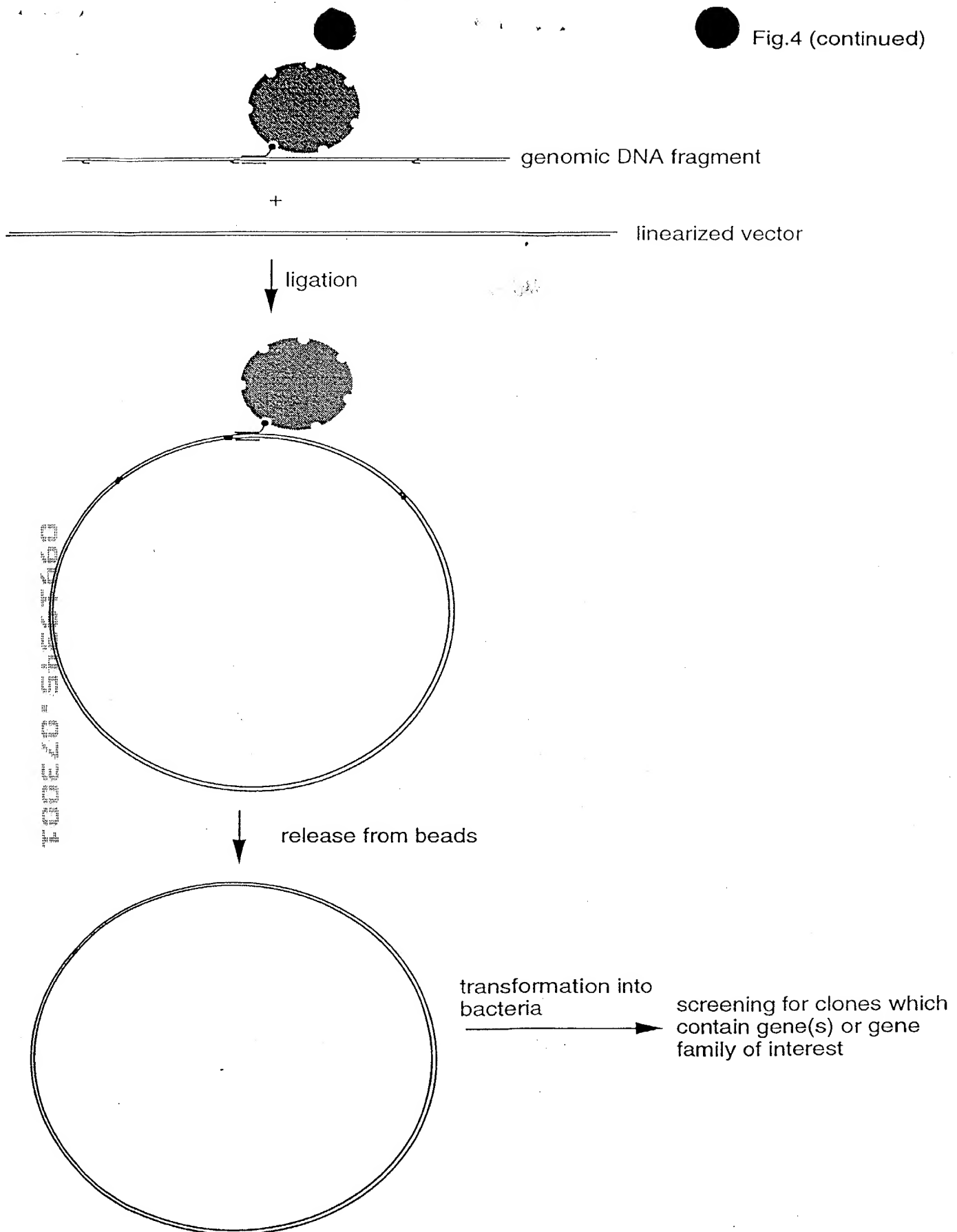


cssDNA probes specific to given gene or gene families



streptavidin-coated beads





fragment of human HPRT gene

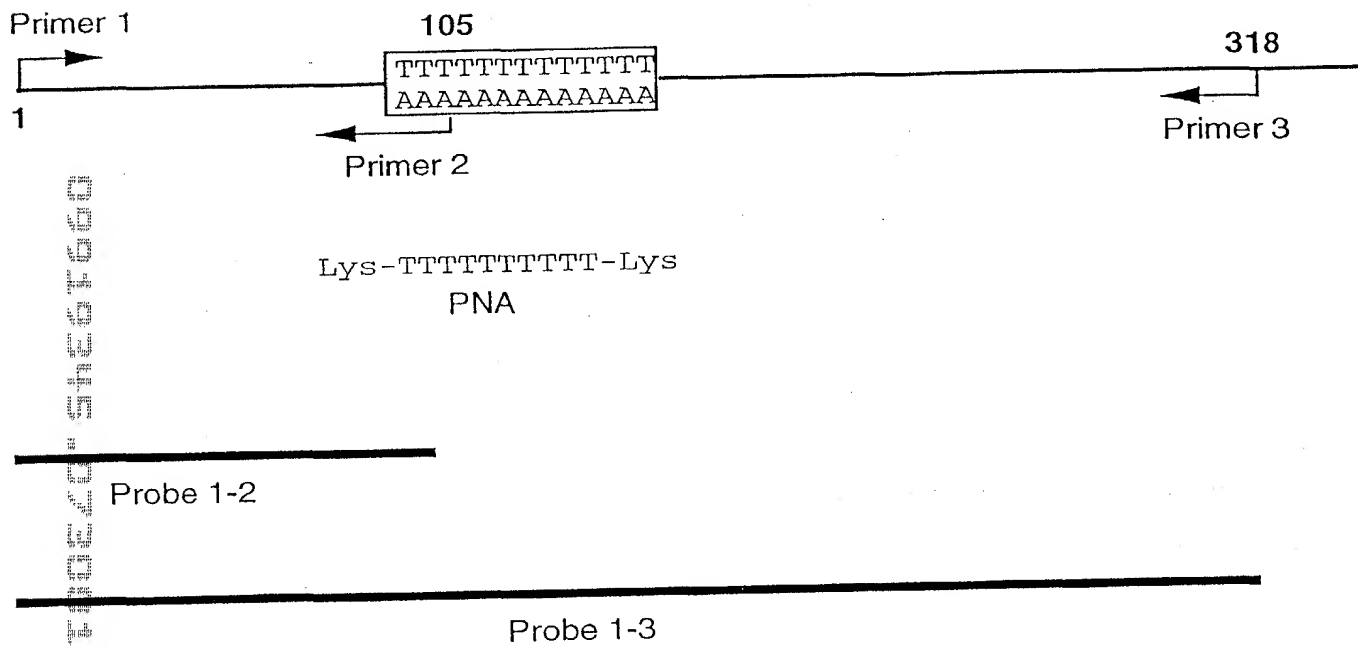


Fig. 5B. Targeting of the human HPRT gene fragment with the probe adjacent to the PNA binding site

Specific target	+				-
PNA	+		-		+
RecA	+	-	+	-	+
lane	1	2	3	4	5

start

Linear target DNA

Free probe

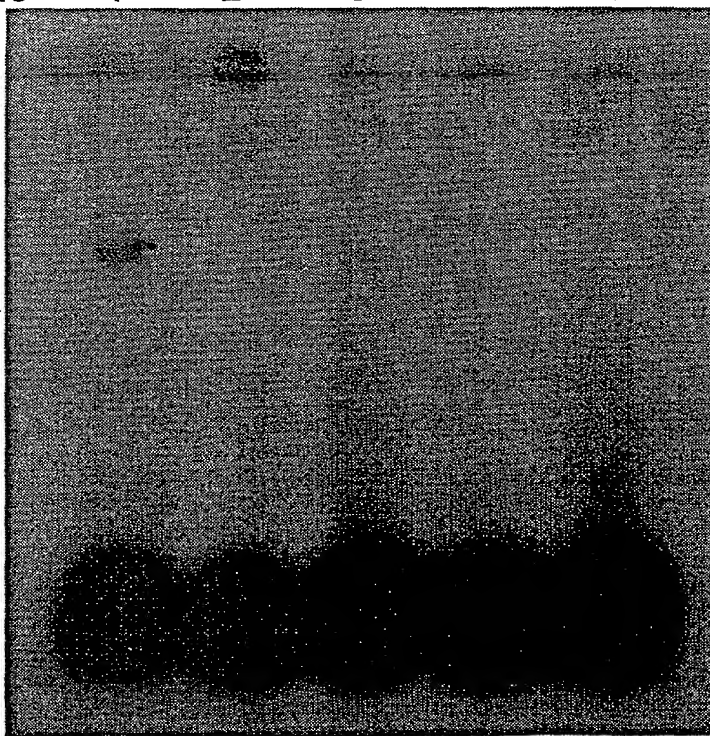
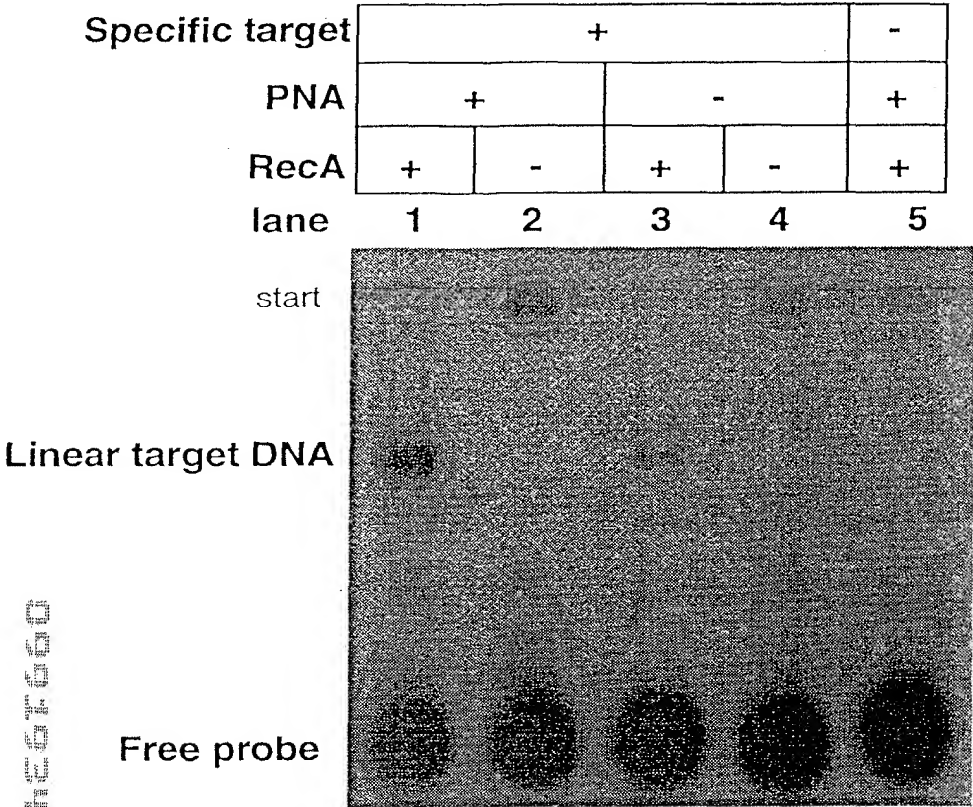


Fig. 5C. Targeting of the human HPRT gene fragment with the probe with the PNA binding site inside it



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